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(54) Title: METHOD FOR PRODUCING BACTERIA FERMENTATIVE PRODUCTS FOR FOOD CONTAINING LACTIC ACID

(57) Abstract: The disclosure concerns a method for producing fermentative media comprising a step of hydrolyzing dairy resources, a fermentative media produced by the above method, and a method for producing bacteria fermentative products for food containing lactic acid. Bacteria fermentative products produced by the method of the present invention contain a large number of lactic acid bacteria; the products can be stored for a long time, and can be used for alimentotherapy.

METHOD FOR PRODUCING BACTERIA FERMENTATIVE PRODUCTS FOR FOOD CONTAINING LACTIC ACID

FIELD OF THE INVENTION

The present invention relates to a method for producing fermentative media comprising a step of hydrolyzing dairy resources, a fermentative media produced by the above method, and a method for producing bacteria fermentative products for food containing lactic acid.

BACKGROUND OF THE INVENTION

Yogurt is basically prepared by inducing lactic acid fermentation in animal milk using *Lactobacillus* and especially when yogurt is produced by inoculating milk with both *Lactobacillus* and *Bifidobacterium* (or by mixing two kinds of fermented milk together - one is produced by *Bifidobacterium*, the other is produced by *Lactobacillus*), it is expected to have various valuable effects peculiar to the above bacteria since it contains both *Lactobacillus* and *Bifidobacterium*, resulting in the increase of consumption. Fermented milk using live bacteria like the above yogurt is widely consumed as a subsidiary health food owing to

its effects of intestinal function control and immune enhancing. In order to keep such physiological effects stable, it is important to maintain useful bacteria as *Lactobacillus* in a state of living and maintain their activity to high level.

When the culture of Lactobacillus for food production in which the flavor of the food is important, proliferation cannot be the best interest for the selection of target bacterium. It is rather better to select bacterium having good flavor, though bad proliferation.

The present inventors have accomplished the present invention by preparing bacteria fermentative products for food containing lactic acid with improved characteristics of treatment and alimentotherapy, and prolonged the term of validity by hydrolyzing dairy resources and pasteurizing the fermentative products.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a method for producing bacteria fermentative media for food containing lactic acid.

It is a further object of the present invention to provide a bacteria fermentative media for food containing lactic acid that is produced by the above

method.

It is an additional object of the present invention to provide a method for producing bacteria fermentative products for food containing lactic acid by using the above bacteria fermentative media for food containing lactic acid.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

To accomplish those objects, the present invention provides a method for producing bacteria fermentative media for food containing lactic acid.

The present invention also provides a bacteria fermentative media for food containing lactic acid that is produced by the above method.

The present invention also provides a method for producing bacteria fermentative products for food containing lactic acid by using the above bacteria fermentative media for food containing lactic acid.

Hereinafter, the present invention is described in detail.

In one aspect, the present invention provides a method for producing bacteria fermentative media for food containing lactic acid comprising a step of hydrolyzing dairy resources, and a bacteria fermentative media for food containing lactic acid

produced by the above method.

The method for producing bacteria fermentative media for food containing lactic acid of the present invention includes following steps:

1) Hydrolyzing dairy resources;

2) Obtaining filtrate or supernatant by filtration or centrifugation after heating the above hydrolysate;

3) Adding materials for the growth of bacteria to the above solution; and

4) Sterilizing the above solution.

It could be possible to add starch to dairy resources before hydrolysis and in that case, it is preferable to add starch in the weight part of 0.1-10 to 100 weight part of dairy resources. It is more preferred to add 0.5-5 weight part of starch, and most preferred to add 1-2 weight part of starch.

Every dairy resource, which is suitable for bacteria fermentative media for food containing lactic acid, can be used for the present invention, and it is preferable to use one or more resources selected from a group consisting of skim milk, milk and soybean milk. Using milk or soybean milk not skim milk can enhance such characteristics as taste, flavor and shape.

Concerning hydrolysis above, it is a step to

hydrolyze dairy resources or both dairy resources and starch together by adding one or more enzymes selected from a group consisting glycolyase, protease and pancreatin. Every glycolyase that is able to decompose sugar can be used for the present invention and especially amylase is preferably used. Every protease or peptidase that is able to decompose protein or polypeptide can be used. When starch is added to dairy resources, glycolyase and protease are ought to be used by turns and their pH level should be regulated to meet 4.0-4.5 using acetic acid followed by pasteurization.

Also, every material that is generally used for the production of bacteria culture medium can be used as a material for the growth of bacteria. Peptone can be used as a nitrogen source and dextrose can be used as a carbohydrate source. Sodium chloride can be used as a mineral source and to control osmotic pressure. Yeast extract can be used to supply vitamins, amino acids and micronutrient elements. Agar is preferably added for bacteria culture of the present invention. Meanwhile, every ingredient that is generally known to be possibly substituted or added for bacteria culture can be used. At this time, it is preferable to add one or more components selected from a group consisting of agar, sodium chloride, yeast extract, peptone, lactose and dextrose.

Hydrolyzed and concentrated amino acid mixture also can be added to the above hydrolysate of the step 1. At this time, it is preferable to add 0.1-10 weight part of hydrolyzed/concentrated amino acid mixture per 100 weight part of hydrolysate and 1-3 weight part of the mixture is more preferred. As a source of amino acid, the above hydrolyzed/concentrated amino acid mixture plays a role in stimulating the growth of *Lactobacillus* and is prepared by hydrolyzing milk serum protein or casein with protease followed by vacuum concentration. The titer of *Lactobacillus* was not increased with less than 0.1 weight part of the hydrolyzed/concentrated amino acid mixture while organoleptic characteristics of food became deteriorated with more than 10 weight part of the mixture.

It is also possible to add one or more components selected from a group consisting of pine needles extract, honey, and mixture of honey and pine needles extract (honey:pine needles extract = 0.5:2.0-1.0:2.0) to the above hydrolysate of the step 1. At this time, the preferable ratio of the hydrolysate to the additional components is 100 weight part to 0.1-10 weight part, and the ratio 100 to 1-3 weight part could be more preferable. The pine needles extract, a

biologically active additive, is a sticky solution having dark color and unique taste and flavor of resin. The pine needles extract, honey, and the mixture of honey and pine needles extract contribute to keeping biologically active *Lactobacillus* alive for a long period (15-18 days) and at the same time show opposite action against micro flora. Therefore, (treatment-alimentotherapeutic) characteristics of fermentative products of the present invention can be enhanced by adding honey, pine needles extract or the mixture of honey and pine needles extract. At this time, if the added amount of those is less than 0.1 weight part to 100 weight part of hydrolysate, they have no effect, and adding more than 10 weight part is proved to be wasteful without any additional effect. When the mixture of honey and pine needles extract is added, the mixing ratio should be carefully regulated. For example, if the honey content is less than 2:0.5, additional effect of the mixture is not enhanced. And if the honey content is more than 2:1, the validity term of fermentative products become shortened comparing to the products with only the extract of pine needles.

Besides, it could be also possible to add extracts of *Ulmus davidiana var. japonica* or *Atractylodes japonica* roots to the above hydrolysate of

the step 1. At this time, the preferable ratio of hydrolysate to those additional extracts is 100 weight part to 1-10 weight part and adding 1-3 weight part of those extracts could be more preferable. The extracts of *Ulmus davidiana var. japonica* and *Atractylodes japonica* roots having medicinal effects on gastrointestinal diseases contribute to keep biologically active *Lactobacillus* alive for a long period (15-18 days) and at the same time they have opposite actions against micro flora. Therefore, it is preferable to add the extracts of *Ulmus davidiana var. japonica* and *Atractylodes japonica* roots since those extracts strengthen the function of fermentative products owing to the unique effect of *Ulmus davidiana var. japonica* and *Atractylodes japonica* roots such as anti-bacterial effect and immune enhancing effect along with their various organic acids. Precisely, *Ulmus davidiana var. japonica* and *Atractylodes japonica* roots have such effects as helping digestion, anticancer effect, immune enhancing effect, and increasing the efficacy of *Lactobacillus*, so that treatment-alimentotherapeutic characteristics, biological value, functional value and sensuous property of the fermentative products of the present invention can be all enhanced by adding the extracts of *Ulmus davidiana var. japonica* and *Atractylodes japonica* roots. Those

enhanced characteristics are further related to the increased resistance against pathogen microorganisms since the added natural herbs have antibiosis. The extracts of *Ulmus davidiana var. japonica* and *Atractylodes japonica* roots have dark brown color and bitter taste. By adding the extracts containing about 12% of solid *Ulmus davidiana var. japonica* and about 25% of solid *Atractylodes japonica* roots, the strong sour taste of *Lactobacillus* culture becomes mild still with original flavor. When they are added with fewer amounts than 0.1 weight part to 100 weight part of hydrolysate, they have no effect and with more than 10 weight part, the taste becomes bitter not to be suitable for food production.

For the sterilization of the above step 4, pasteurization at a low temperature is preferable. Precisely, pasteurization at 110-118℃ for 10-60 minutes is preferable and pasteurization at 115℃ for 20-30 minutes is more preferable.

In the preferred embodiments of the present invention, a method for producing bacteria fermentative media for food containing lactic acid includes following steps:

1) Add 0.5-5 weight part of starch to 100 weight part of dairy resources followed by stirring, after

which heat thereof at 95-105℃ for 5-60 minutes;

2) Control the pH of the above solution to 6.0-8.0 and cool thereof at 75-85℃, after which add 0.001-0.003 weight part of amylase thereto;

3) Cool the above solution to 45-50℃, after which add 0.05-0.2 weight part of protease thereto followed by hydrolyzing;

4) Control the pH of the above solution to 4.4-4.6 and then heat thereof at 95-105℃ for 10-20 minutes, after which remove precipitates by filtration or centrifugation to obtain supernatants;

5) Add 0.5-1.5 volumes of distilled water to the above supernatants for dilution, and then add materials necessary for the growth of bacteria thereto; and

6) Sterilize the above media.

In the preferred embodiments of the present invention, a method for producing bacteria fermentative media for food containing lactic acid includes following steps:

1) Stir the dairy resources, after which heat thereof at 95-105℃ for 5-60 minutes;

2) Cool the above solution to 40-50℃, after which control the pH to the level of 7.5-8.5;

3) Add 0.05-0.2 weight part of pancreatin to the above solution followed by hydrolyzing;

4) Control the pH of the above hydrolysate to the level of 4.4-4.6, and then heat thereof at 95-105℃ for 10-20 minutes, after which remove precipitates by filtration or centrifugation to obtain supernatants;

5) Add 0.5-1.5 volumes of distilled water to the above supernatants for dilution, and then add materials necessary for the growth of bacteria thereto; and

6) Sterilize the above media.

The present invention also provides a method for producing bacteria fermentative products for food containing lactic acid using the above bacteria fermentative media for food containing lactic acid.

At first, inoculated *Lactobacillus* at 37.5~38℃ in vitro and then left thereof for a day, after which inoculated the above prepared fermentative media with the grown inoculum. The preferable amount of inoculum for inoculation is 0.1-15 weight parts per 100 weight parts of fermentative media and 3-10 weight part per 100 weight parts of fermentative media could be more preferable.

For the inoculum of the present invention, *Bifidobacteria* and/or *Lactobacteria* and/or *Streptococus thermophilus* and/or their daily culture can be used and for bacteria strains, one or more bacteria strain selected from a group consisting of *Lactobacterium*

plantarum 8 PA3, *Lactobacter plantarum* 296, *Lactobacillus acidophilus*, *Bifidum longum* 379 M, *Bifidum* 791, *Bifidum* 1, *Streptococcus thermophills*, *Lactobacillus plantarum* (KCTC1048), *L. acidophilus* (KCTC 3111), *Bifidobacterium bifidum* (KCTC 3202, 3357), *B. longum* (KCTC 3421, 3128), *Streptococcus thermophilus* (KCTC 3658), *Bifidobacterium longum* 379M ПарТИЯ1 ОТ 11.00.01r., *Lactobacillus acidophilus* ПарТИЯ2 ОТ 10.02.01r. provided from Russia can be used solely and together.

In order to produce the bacteria fermentative products for food containing lactic acid of the present invention, the present inventors inoculated the above prepared bacteria fermentative media for food containing lactic acid with bacteria, followed by culturing thereof in a fermentor at 35-38℃ for 10-25 hours.

The present invention further provides fermentative products for food containing lactic acid prepared by the above method.

The fermentative products for food containing lactic acid produced by the method of the present invention supply nutrition without any breakdown owing to the pasteurization at low temperature have much larger number of Lactobacillus than other fermentative products do and can be stored for a long time. In

addition, the producing method of the present invention can be used in the milk or soybean milk manufacturing industry.

EXAMPLES

Practical and presently preferred embodiments of the present invention are illustrative as shown in the following Examples.

However, it will be appreciated that those skilled in the art, on consideration of this disclosure, may make modifications and improvements within the spirit and scope of the present invention.

Example 1: Preparation of bacteria fermentative products for food containing lactic acid 1

1. Added 150 g of starch to 15 ℓ of skim milk followed by stirring. Then, heated thereof at 100℃ for 30 minutes. Overheating (either over temperature or over time) causes color change of skim milk and bad property. On the contrary, heating time and/or temperature is not sufficient, the reaction time and the amount of enzyme are required more.

2. Regulated pH thereof to the level of 6.0-7.0 using 20-40% edible NaOH solution and acetic acid. When the temperature reached 80℃, added 0.3 g of amylase (0.2% of starch) having strong heat-resistance

thereto. As enzyme reaction started, operated cooler to cool it down to 48℃ (about 50 minutes were required).

Unlike the conventional method, it was possible to produce various prebiotics such as dextrin, branched oligosaccharide, and maltotriose promoting the growth of probiotics by taking advantage of amylase having strong heat resistance and adding starch. And maximized the usage of time and temperature by using the cooling period from high temperature.

3. When the temperature cooled down to 48℃, added 15 g of protease thereto. And then, hydrolysis was performed at 48℃, pH 6.5-7.5 for 1 hour to obtain hydrolysate.

The enzyme reaction process took 4 hours with pancreatin but it could be shortened by about 3 hours with protease. Besides, inactivation process was not required when protease was added since protease suppressed excessive enzyme reaction of amylase.

4. One hour after the reaction with protease, regulated the pH of the solution to 4.5±0.1 using acetic acid, and then boiled thereof for 15 minutes followed by filtration or centrifugation, resulting in obtaining clear filtrate or supernatant (hydrolysate of skim milk). If the pH level was out of the range 4.5± 0.1, precipitation was not generated well, or filtrate

or supernatant became unclear.

5. Diluted the above hydrolysate with distilled water at the ratio of 1:1 and then added 22.5 g of starch, 150 g of sodium chloride, 80 g of yeast extract and 3 g of cysteine.

6. Sterilized thereof at 0.7 air pressure for 45 minutes after warming thereof for 30 minutes with steam.

If the sterilizing time or temperature is not sufficient, it is difficult to expect complete sterilization and the color of media becomes unclear. On the other hand, if the sterilizing time or temperature is excessive, the media shows very dark color and may stink.

7. Cooled the sterilized media to 36-40℃ (optimum temperature range of inoculum).

8. Inoculated the sterilized media with daily culture of *Bifidobacterium bifidum* (KCTC 3202) at the ratio of 100 weight parts of media to 3 weight parts.

9. Produced fermentative products by culturing thereof 37℃ for 12 hours.

10. The produced fermentative products have sour taste and smell like oxidized milk. As sticky liquid having brown color, the fermentative products contain more than 10^9/㎖ *Lactobacillus* and can be stored over 3 weeks at 4℃ without losing their primary characteristics.

Example 2: Preparation of bacteria fermentative products for food containing lactic acid 2

The present inventors have produced the bacteria fermentative products with the same method as the above Example 1 except inoculating daily culture of *Lactobacillus plantarum* (KCTC 1048) at the ratio of 100 weight part of skim milk-hydrolysate to 3 weight part.

Example 3: Preparation of bacteria fermentative products for food containing lactic acid 3

The present inventors have produced the bacteria fermentative products with the same method as the above Example 1 except inoculating daily culture of *Streptococcus thermophilus* (KCTC 3658) at the ratio of 100 weight part of skim milk-hydrolysate to 3 weight part.

Experimental Example 1: Analysis of characteristics of fermentative products 1

The present inventors have tested the characteristics of the fermentative products produced in the above Example 1-3 with following steps. The results were summarized in the Table 1.

1. Concentration of *Lactobacillus*

The concentration of Lactobacillus represented

the number of bacteria cells. Cells per 1 ml of fermentative products were measured by 10-fold dilution method.

2. Cell morphology

The cells of fermentative products were gram stained and observed with a microscope.

3. Additional micro flora

In order to confirm if other bacteria except those used as inoculum were included, the present inventors checked colonies using general nutrient media and plate count agar, and observed them with a microscope.

4. Preservation term of fermentative properties

Preservation term of fermentative properties means a period during which *Lactobacillus* of fermentative products can still be used as a seed for the next fermentation. During this period, the growth and fermentative power of *Lactobacillus* of fermentative products are kept in a great condition.

The fermentative products were taken as samples and then kept in cold storage. Each sample was used as a seed to prepare fermentative products and tested to see if they still had primary characteristics of fermentative products (bacteria concentration, cell morphology, growth rate, etc).

5. Term of validity

The period of circulation (term of validity) of the fermentative products was determined by surveying the properties, cell number, cell morphology thereof periodically while keeping the products in cold storage.

5. Properties

Taste, color and flavor of the fermentative products were evaluated by selected inspectors with 5-grade evaluation method (5.0~4.6 : Fermentative products had sour taste. Unique taste and flavor of additives were harmonized well with those of the fermentative products., 4.5~4.1 : Fermentative products had sour taste and also unique taste and flavor of additives were still alive., 4.09~3.6 : Fermentative products had sour taste and unique taste and flavor of additives became mild., Below 3.5 : Fermentative products had sour taste. Taste and flavor were not changed.).

<Table 1>

Characteristics of the fermentative products produced in Example 1-3

Index	Example 1 (KCTC3202)	Example 2 (KCTC1048)	Example 3 (KCTC3658)
Concentration of *Lactobacillus* (Titer)	6.5×10^9	6.7×10^9	6.9×10^9

Morphology of cells	General	General	General
Existence of additional Micro flora	No	No	No
Term of preservation (day)	2-3	3	2-3
Term of validity (day)	15-18	15-18	15-18
Taste	3.9	3.9	3.9

Example 4: Preparation of bacteria fermentative products for food containing lactic acid 4

The bacteria fermentative products for food containing lactic acid were produced as follows.

1. Boiled skim milk for 2~3 minutes.

2. Cooled it down to 45℃.

3. Regulated the pH thereof to the level of 8.0 using 10~20% edible Na solution.

4. Hydrolyzed thereof for 4 hours with pancreatin while the pH level was maintained at 8.0±0.2

5. Added 1~2% chloroform.

6. Maintained the temperature thereof at 37℃ for 14~16 hours.

7. Regulated the pH thereof to the level of 4.5± 0.1 using 30% acetic acid.

8. Boiled thereof for 15 minutes.

9. Filtered thereof.

10. Stored the above obtained hydrolysate for 6~8 months with chloroform (1% volume) and used thereof whenever needed. Later, diluted the hydrolysate with water at the ratio of 1:1, after which added sodium chloride and peptone thereto. Heated thereof to 80℃ and then mixed with agar.

11. After boiled the above mixture for 15 minutes, added lactose, cystein or soluble hydrochloride cystein thereto, followed by sterilization at 0.5 air pressure for 30 minutes.

The components of the skim milk-hydrolysate were summarized in Table 2.

<Table 2>

Component	Content
Skim milk	500 ㎖
Distilled water	500 ㎖
Sodium chloride	Up to 5% (Considered chloride contained in hydrolysate)
Lactose	10 g
L-systein [hydrochloride]	100 ㎎
Peptone	2 g
Agar	750 ㎎

12. After sterilization, added daily culture of *Bifidobacterium bifidum* (KCTC 3202) with the amount of 3 weight part to 100 weight part of skim milk-hydrolysate. The pH of the prepared hydrolysate was 7.2-7.4.

13. Cultured thereof at 37℃ for 18~20 hours. The obtained fermentative products were light brown colored solution having sour taste. The fermentative products contained 10^7~10^8 living bacteria per 1 ㎖.

Experimental Example 2: Analysis of characteristics of fermentative products

The characteristics of the fermentative products produced in Example 1 were compared with those produced in Example 4, and the results were shown in Table 3.

<Table 3>

Index	Example 1	Example 4
Concentration of *Lactobacillus* (titer)	6.5×10^9	2.4×10^8
Cell morphology	General	Degenerated
Additional Micro flora	No	No
Term of preservation (day)	2-3	1
Term of validity (day)	15-18	6-8

Culture time (hour)	12-13	18-20
Taste	Sour	Sour

The fermentative products produced in Example 4 contained degenerated-shaped *Lactobacillus*, while those produced in Example 1 contained general-shaped *Lactobacillus*. And the concentration of *Lactobacillus* of the fermentative products produced in Example 1 was about 10^9/㎖, which was at least 10 times more than that of the fermentative products produced in Example 4. It was something meaningful. Especially, it affected on prolonging the preservation term of fermentative properties by 1~2 more days, and the validation term by 8~12 days.

Treatment-alimentotherapeutic characteristics of the fermentative products were also strengthened. It is important for food containing *Lactobacillus* to keep certain level of living *Lactobacillus* in order to work effectively in the intestines. And thus, fermentative products with increased concentration of *Lactobacillus* of the present invention can be effectively used in variety.

The culture time of the process in Example 1 was at least shorter than that of the process in Example 4, suggesting that shorter culture time can contribute to the cost reduction and strengthening competitiveness by

simplified manufacturing process. Moreover, the total preparation time from the beginning to the last step of obtaining final fermentative products in Example 1 was at least 9 hours shorter, comparing to the Example 4.

Example 5: Preparation of bacteria fermentative products for food containing lactic acid 5

The present inventors have produced the bacteria fermentative products with the same method as the above Example 1 except inoculating skim milk-hydrolysate with daily culture of *Bifidobacterium bifidum* (KCTC 3202) at the ratio of 100 weight part of skim milk-hydrolysate to 10 weight part of daily culture, and shortening the culture time by 4~6 hours at 37℃.

Fermentative products produced in this Example 5 had strong sour taste and contained more than 7.2×10^{10}/㎖ *Bifidobacteria*. The validation term of the fermentative products was over 10 days.

Example 6: Preparation of bacteria fermentative products for food containing lactic acid 6

The present inventors have produced the bacteria fermentative products with the same method as the above Example 1 except inoculating skim milk-hydrolysate with daily culture of *Lactobacillus plantarum* (KCTC 1048) at the ratio of 100 weight part of skim milk-hydrolysate

to 10 weight part of daily culture, and shortening the culture time by 4 hours at 37℃.

Fermentative products produced in this Example 6 had strong sour taste and contained more than 8.8×10^{10}/㎖ *Lactobacteria*. The validation term of the fermentative products was over 10 days.

Example 7: Preparation of bacteria fermentative products for food containing lactic acid 7

The present inventors have produced the bacteria fermentative products with the same method as the above Example 1 except inoculating skim milk-hydrolysate with daily culture of *Streptococcus thermophilus* (KCTC 3658) at the ratio of 100 weight part of skim milk-hydrolysate to 10 weight part of daily culture, and shortening the culture time by 4 hours at 37℃.

Fermentative products produced in this Example 5 had strong sour taste and contained more than 6.9×10^{10}/㎖ *Streptococcus thermophilus*. The validation term of the fermentative products was over 10 days.

Experimental Example 3: Analysis of characteristics of fermentative products

The present inventors have tested the characteristics of the fermentative products produced in Example 5-7. The results were summarized in Table 4.

<Table 4>

Index	Example 5 (KCTC3202)	Example 6 (KCTC1048)	Example 7 (KCTC3658)
Concentration of *Lactobacillus* (Titer)	7.2×10^{10}	8.8×10^{10}	6.9×10^{10}
Cell morphology	General	General	General
Additional micro flora	No	No	No
Culture time (hour)	8-10	7-8	8-10
Term of preservation (day)	2	2	2-3
Term of validity (day)	10-13	10-12	10-13
Taste	Sour	Sour	Sour

Example 8: Preparation of bacteria fermentative products for food containing lactic acid 8

The present inventors have produced the bacteria fermentative products with the same method as the above Example 1 except adding 300 g of starch into 15 ℓ of skim milk.

Example 9: Preparation of bacteria fermentative

products for food containing lactic acid 9

The present inventors have produced the bacteria fermentative products with the same method as the above Example 2 except adding 300 g of starch into 15ℓ of skim milk.

Example 10: Preparation of bacteria fermentative products for food containing lactic acid 10

The present inventors have produced the bacteria fermentative products with the same method as the above Example 3 except adding 300 g of starch into 15ℓ of skim milk.

Example 11-13: Preparation of bacteria fermentative products for food containing lactic acid 11-13

The present inventors have produced the bacteria fermentative products with the same method as the above Example 1, 2 and 3, respectively. At this time starch was not added.

The characteristics of the fermentative products produced in Example 1-3, 8-10 and 11-13 were tested. The results were summarized in Table 5.

<Table 5>

Change of pH and final cell concentration according to the culture time increasing by starch

Culture time	Example 11	Example 1	Example 8	Example 12	Example 2	Example 9	Example 13	Example 3	Example 10
	Amounts of added starch (weight part) to 100 weight part of skim milk								
	0	1	2	0	1	2	0	1	2
0	6.25	6.25	6.25	6.20	6.21	6.20	6.25	6.24	6.26
2	6.21	6.12	6.18	6.15	6.09	6.15	6.22	6.16	6.19
4	6.06	5.83	5.99	5.94	5.77	5.89	6.01	5.82	5.97
6	5.76	5.29	5.48	5.62	5.27	5.58	5.77	5.36	5.66
8	5.38	4.68	5.02	5.22	4.72	5.14	5.39	4.77	5.23
10	4.96	4.21	4.78	4.89	4.16	4.75	4.99	4.24	4.88
12	4.62	3.94	4.35	4.44	3.91	4.35	4.67	4.01	4.44
14	4.31	3.89	4.11	4.23	3.86	4.12	4.36	4.00	4.20
16	4.08	3.87	4.01	4.05	3.85	4.02	4.20	3.99	4.08
18	4.02	3.86	4.00	3.89	3.84	3.97	4.02	3.99	4.03
Final cell conc.	2.2×10^8	6.8×10^9	7.2×10^8	$3.8\ 10^8$	6.0×10^9	8.5×10^8	2.7×10^8	6.2×10^9	6.6×10^8

From the results of the above experiments, it was confirmed that there were big differences in the growth of bacteria (change of pH) according to culture time and the final cell concentrations of fermentative products when starch was added.

Precisely, when starch was added at the ratio of 1 weight part to 100 weight part of skim milk, fermentative characteristics was proved to be excellent but when starch was added less than 1 weight part, fermentative characteristics was not affected thereby.

Adding 2 weight part of starch also showed better

fermentative characteristics but not so much as 1 weight part of starch was added. In the meantime, when more than 2 weight part of starch was added, it became lumped together, by which enzyme was hindered to penetrate. Therefore, it was not preferable to add more than 2 weight part of starch because it leaded to the increase of unhydrolyzed solid body.

The pH level relates directly to the growth of bacteria since *Lactobacillus* generates lactic acid as being growing resulting in lowering pH level. Therefore, the growth of bacteria could be measured by observing the change of pH level. However, rapid drop-down of pH level could reversely effect on *Lactobacillus*. Thus, excessive fermentation should be avoid and it is recommended to finish culturing at pH level pH 4.0±0.1.

Example 14-18: Preparation of bacteria fermentative products for food containing lactic acid 14-18

The present inventors have produced the bacteria fermentative products with the same method as the above Example 1 except adding 0.5, 1, 2, 3, 3.5 weight part of pine needles extract to 100 weight part of skim milk-hydrolysate before sterilizing of the hydrolysate.

The fermentative products produced in Example 14-18 tasted a bit sour and bitter, and smelled like tree

resin. As shown in Table 6, the fermentative products showed preferable characteristics when 1-3 weight part of pine needles extract was added.

<Table 6>

Characteristics of the fermentative products produced in Example 14-18

Index	Amounts of added pine needles extract (weight part) to 100 weight part of hydrolysate				
	Example 14	Example 15	Example 16	Example 17	Example 18
	0.5	1	2	3	3.5
Concentration of *Lactobacillus* (titer)	5.5×10^{9}	8.6×10^{9}	9.4×10^{9}	4.2×10^{9}	8.4×10^{7}
Cell morphology	General	General	General	Degenerated, general	Degenerated
Additional micro flora	No	No	No	No	No
Term of preservation (day)	3	3-4	3-4	3-4	2
Term of validity (day)	15-17	18	18	18	10
Taste	Sour and bitter	Sour and bitter	Sour and bitter	Sour and bitter	Sour and bitter

Example 19-23: Preparation of bacteria fermentative products for food containing lactic acid 19-23

The present inventors have produced the bacteria fermentative products with the same method as the above Example 2 except adding 0.5, 1, 2, 3, 3.5 weight part of pine needles extract to 100 weight part of skim milk-hydrolysate before sterilizing of the hydrolysate.

The fermentative products produced in Example 19-23 tasted a bit sour and bitter, and smelled like tree resin. As shown in Table 7, the fermentative products showed preferable characteristics when 1-3 weight part of pine needles extract was added.

<Table 7>

Characteristics of the fermentative products produced in Example 19-23

Index	Amounts of added pine needles extract (weight part) to 100 weight part of hydrolysate				
	Example 19	Example 20	Example 21	Example 22	Example 23
	0.5	1	2	3	3.5
Concentration of *Lactobacillus* (titer)	6.0×10^{9}	8.2×10^{9}	8.4×10^{9}	1.2×10^{10}	3.8×10^{8}
Cell morphology	General	General	General	General	Degenerated, General
Additional micro flora	No	No	No	No	No
Term of preservation (day)	3	4	4	4	3

Term of validity (day)	15	18	18	18	12
Taste	Sour and bitter	Sour and bitter	Sour and bitter	Sour and bitter	Sour and bitter

Example 24-28: Preparation of bacteria fermentative products for food containing lactic acid 24-28

The present inventors have produced the bacteria fermentative products with the same method as the above Example 3 except adding 0.5, 1, 2, 3, 3.5 weight part of pine needles extract to 100 weight part of skim milk-hydrolysate before sterilizing of the hydrolysate.

The fermentative products produced in Example 24-28 tasted a bit sour and bitter, and smelled like tree resin. As shown in Table 8, the fermentative products showed preferable characteristics when 1-3 weight part of pine needles extract was added.

<Table 8>

Characteristics of the fermentative products produced in Example 24-28

Index	Amounts of added pine needles extract (weight part) to 100 weight part of hydrolysate				
	Example 24	Example 25	Example 26	Example 27	Example 28
	0.5	1	2	3	3.5

Concentration of *Lactobacillus* (titer)	2.2×10^{9}	2.4×10^{9}	6.4×10^{9}	2.5×10^{10}	8.6×10^{8}
Cell morphology	General	General	General	General	Degenerated, General
Additional Micro flora	No	No	No	No	No
Term of preservation (day)	3	3	3	4	2-3
Term of validity (day)	15	17	17	18	18
Taste	Sour and bitter	Sour and bitter	Sour and bitter	Sour and bitter	Sour and bitter

Example 29-33: Preparation of bacteria fermentative products for food containing lactic acid 29-33

The present inventors have produced the bacteria fermentative products with the same method as the above Example 1 except adding 0.5, 1, 2, 3, 3.5 weight part of the mixture of pine needles extract and honey (pine needles extract:honey = 2 : 0.5 weight part) to 100 weight part of skim milk-hydrolysate before sterilizing of the hydrolysate.

Example 34-35: Preparation of bacteria fermentative products for food containing lactic acid 34-35

The present inventors have produced the bacteria fermentative products with the same method as the above Example 1 except adding 1 and 3 weight part of honey to

100 weight part of skim milk-hydrolysate before sterilizing of the hydrolysate.

The characteristics of the fermentative products produced in Example 29-35 were tested and the results were summarized in Table 9.

<Table 9>

Characteristics of the fermentative products produced in Example 29-35

Index	Amounts of added mixture of pine needles extract and honey (weight part) to 100 weight part of hydrolysate			Amounts of added honey (weight part) to 100 weight part of hydrolysate			
	Example 29	Example 30	Example 31	Example 32	Example 33	Example 34	Example 35
	0.5	1	2	3	3.5	1	3
Concentration of *Lactobacillus*	5.6×10^{9}	4.8×10^{9}	3.1×10^{10}	3.3×10^{10}	1.5×10^{9}	3.4×10^{9}	5.7×10^{9}
Cell morphology	General	General	General	General	General	General	General
Additional micro flora	No	No	No	No	No	No	No
Term of preservation (day)	3	3	4	4	2-3	2-3	3
Term of validity (day)	17	16	17	17	16	16	17

Taste	Sour, bitter and sweet	Sour and sweet	Sour and sweet	Sour and sweet	Sour and sweet	Sour and sweet	Sour and sweet

Example 36-40: Preparation of bacteria fermentative products for food containing lactic acid 36-40

The present inventors have produced the bacteria fermentative products with the same method as the above Example 2 except adding 0.5, 1, 2, 3, 3.5 weight part of the mixture of pine needles extract and honey (pine needles extract:honey = 2 : 0.5 weight part) to 100 weight part of skim milk-hydrolysate before sterilizing of the hydrolysate.

Example 41-42: Preparation of bacteria fermentative products for food containing lactic acid 41-42

The present inventors have produced the bacteria fermentative products with the same method as the above Example 2 except adding 1 and 3 weight part of honey to 100 weight part of skim milk-hydrolysate before sterilizing of the hydrolysate.

The characteristics of the fermentative products produced in Example 36-42 were tested and the results were summarized in Table 10.

<Table 10>

Characteristics of the fermentative products produced

in Example 36-42

Index	Amounts of added mixture of pine needles extract and honey (weight part) to 100 weight part of hydrolysate			Amounts of added honey (weight part) to 100 weight part of hydrolysate			
	Example 36	Example 37	Example 38	Example 39	Example 40	Example 41	Example 42
	0.5	1	2	3	3.5	1	3
Concentration of *Lactobacillus*	3.8×10^{9}	2.0×10^{10}	2.8×10^{10}	3.3×10^{10}	2.9×10^{9}	5.4×10^{9}	4.1×10^{10}
Cell morphology	General	General	General	General	General	General	General
Additional micro flora	No	No	No	No	No	No	No
Term of preservation (day)	3	3-4	4	4	3	3	3
Term of validity (day)	17	16	18	18	16	16	18
Taste	Sour, bitter and sweet	Sour and sweet	Sour and sweet	Sour and sweet	Sour and sweet	Sour and sweet	Sour and sweet

Example 43-47: Preparation of bacteria fermentative products for food containing lactic acid 43-47

The present inventors have produced the bacteria fermentative products with the same method as the above Example 3 except adding 0.5, 1, 2, 3, 3.5 weight part

of the mixture of pine needles extract and honey (pine needles extract:honey = 2 : 0.5 weight part) to 100 weight part of skim milk-hydrolysate before sterilizing of the hydrolysate.

Example 48-49: Preparation of bacteria fermentative products for food containing lactic acid 48-49

The present inventors have produced the bacteria fermentative products with the same method as the above Example 3 except adding 1 and 3 weight part of honey to 100 weight part of skim milk-hydrolysate before sterilizing of the hydrolysate.

The characteristics of the fermentative products produced in Example 43-49 were tested and the results were summarized in Table 11.

<Table 11>

Characteristics of the fermentative products produced in Example 43-49

Index	Amounts of added mixture of pine needles extract and honey (weight part) to 100 weight part of hydrolysate			Amounts of added honey (weight part) to 100 weight part of hydrolysate			
	Example 43	Example 44	Example 45	Example 46	Example 47	Example 48	Example 49
	0.5	1	2	3	3.5	1	3

Concentration of *Lactobacillus*	5.5×10^9	6.5×10^9	8.2×10^9	5.4×10^{10}	2.2×10^9	2.2×10^{10}	8.7×10^9
Cell morphology	General	General	General	General	General	General	General
Additional micro flora	No	No	No	No	No	No	No
Term of preservation (day)	2-3	3	3	2-3	3	2-3	3
Term of validity (day)	17	17	17	18	16	18	16
Taste	Sour, bitter and sweet	Sour and sweet	Sour and sweet	Sour and sweet	Sour and sweet	Sour and sweet	Sour and sweet

In the preferred embodiments of the present invention, the preferable mixing ratio of honey and pine needles extract is 0.5:2.0~1.0:2.0. The treatment-alimentotherapeutic characteristics of the fermentative products of the present invention can be enhanced by adding 1~3 weight part of the mixture of honey and pine needles extract to 100 weight part of hydrolysate.

Example 50-54: Preparation of bacteria fermentative products for food containing lactic acid 50-54

The present inventors have produced the bacteria fermentative products with the same method as the above

Example 1 except adding 0.5, 1, 2, 3, and 3.5 weight part of hydrolyzed/concentrated amino acid mixture to 100 weight part of skim milk-hydrolysate before sterilizing of the hydrolysate.

At this time, the hydrolyzed/concentrated amino acid mixture was prepared as follows:

1. Milk serum protein was hydrolyzed with protease (pH 6.5-7.0, 48℃, 1 hour), and regulated the pH thereof to 4.5. Centrifugation was performed to remove precipitates. Regulated the pH thereof to 8.0 to develop precipitates.

2. Centrifugation was performed again to remove supernatants. The obtained precipitates were dried to prepare hydrolyzed/concentrated amino acid mixture.

The fermentative products produced in Example 50-54 were tested, and the results were summarized in Table 12.

<Table 12>

Characteristics of the fermentative products produced in Example 50-54.

Index	Amounts of added hydrolyzed/concentrated amino acid mixture (weight part) to 100 weight part of hydrolysate				
	0.5	1	2	3	3.5
	Example 50	Example 51	Example 52	Example 53	Example 54

Concentration of *Lactobacillus* (titer)	2.3×10^9	2.6×10^{10}	3.8×10^{10}	7.9×10^9	5.2×10^8
Cell morphology	General	General	General	General	General
Additional micro flora	No	No	No	No	No
Term of preservation (day)	2-3	4	4	2-3	2
Term of validity (day)	14	18	18	16	15
Taste	Sour and bitter	Sour and bitter	Sour and bitter	Sour and bitter	Sour and bitter

Example 55-59: Preparation of bacteria fermentative products for food containing lactic acid 55-59

The present inventors have produced the bacteria fermentative products with the same method as the above Example 2 except adding 0.5, 1, 2, 3, and 3.5 weight part of hydrolyzed/concentrated amino acid mixture to 100 weight part of skim milk-hydrolysate before sterilizing of the hydrolysate.

The fermentative products produced in Example 55-59 were tested, and the results were summarized in Table 13.

<Table 13>

Characteristics of the fermentative products produced in Example 55-59.

Index	Amounts of added hydrolyzed/concentrated amino acid mixture (weight part) to 100 weight part of hydrolysate				
	0.5	1	2	3	3.5
	Example 55	Example 56	Example 57	Example 58	Example 59
Concentration of *Lactobacillus* (titer)	3.4×10^9	6.2×10^9	5.8×10^9	6.6×10^9	3.5×10^9
Cell morphology	General	General	General	General	General
Additional micro flora	No	No	No	No	No
Term of preservation (day)	3	3	3	3	3
Term of validity (day)	14	16	16	15	15
Taste	Sour and bitter	Sour and bitter	Sour and bitter	Sour and bitter	Sour and bitter

Example 60-64: Preparation of bacteria fermentative products for food containing lactic acid 60-64

The present inventors have produced the bacteria fermentative products with the same method as the above Example 3 except adding 0.5, 1, 2, 3, and 3.5 weight part of hydrolyzed/concentrated amino acid mixture to 100 weight part of skim milk-hydrolysate before sterilizing of the hydrolysate.

The fermentative products produced in Example 60-64 were tested, and the results were summarized in

Table 14.

<Table 14>

Characteristics of the fermentative products produced in Example 60-64.

Index	Amounts of added hydrolyzed/concentrated amino acid mixture (weight part) to 100 weight part of hydrolysate				
	0.5	1	2	3	3.5
	Example 60	Example 61	Example 62	Example 63	Example 64
Concentration of *Lactobacillus* (titer)	2.2×10^9	6.8×10^9	2.0×10^{10}	8.6×10^9	2.3×10^9
Cell morphology	General	General	General	General	General
Additional micro flora	No	No	No	No	No
Term of preservation (day)	3	3	4	3	3
Term of validity (day)	14	16	17	16	15
Taste	Sour and bitter	Sour and bitter	Sour and bitter	Sour and bitter	Sour and bitter

In the present invention, it was preferable to use hydrolyzed/concentrated amino acid mixture plays a role in stimulating the growth of *Lactobacillus* as an amino acid source.

The above hydrolyzed/concentrated amino acid

mixture was added at the ratio of 1-3 weight part to 100 weight part of hydrolysate. The titer of *Lactobacillus* was not increased with less than 1 weight part of the hydrolyzed/concentrated amino acid mixture while organoleptic characteristics of food became deteriorated with more than 3 weight part of the mixture.

Example 65-67: Preparation of bacteria fermentative products for food containing lactic acid 65-67

The present inventors have produced the bacteria fermentative products with the same method as the above Example 1 except adding 1, 2, 3 weight part of *Ulmus davidiana var. japonica* extract to 100 weight part of skim milk-hydrolysate before sterilizing of the hydrolysate.

The fermentative products produced in Example 65-67 had a decreased sour taste and a slightly increased bitter taste.

Example 68-70: Preparation of bacteria fermentative products for food containing lactic acid 68-70

The present inventors have produced the bacteria fermentative products with the same method as the above Example 1 except adding 1, 2, 3 weight part of *Atractylodes japonica* root extract to 100 weight part

of skim milk-hydrolysate before sterilizing of the hydrolysate.

The fermentative products produced in Example 68-70 had a decreased sour taste and a slightly increased bitter and astringent taste. The characteristics of the fermentative products produced in Example 68-70 were tested and the results were summarized in Table 15.

<Table 15>

Characteristics of the fermentative products produced in Example 65-70

Index	Amounts of added extracts (weight part) to 100 weight part of hydrolysate					
	Extract of *Ulmus davidiana var. japonica*			Extract of *Atractylodes japonica* root		
	1	2	3	1	2	3
	Example 65	Example 66	Example 67	Example 68	Example 69	Example 70
Concentration of *Lactobacillus* (titer)	2.4×10^{9}	3.8×10^{10}	8.7×10^{9}	5.8×10^{10}	3.0×10^{10}	2.5×10^{10}
Cell morphology	General	General	General	General	General	General
Additional micro flora	No	No	No	No	No	No
Term of preservation (day)	3	3-4	2	4	3-4	3-4

Term of validity (day)	17	18	12	20	18	18
Taste, flavor	4.2	4.7	4.2	4.0	4.0	4.0

Example 71-73: Preparation of bacteria fermentative products for food containing lactic acid 71-73

The present inventors have produced the bacteria fermentative products with the same method as the above Example 2 except adding 1, 2, 3 weight part of *Ulmus davidiana var. japonica* extract to 100 weight part of skim milk-hydrolysate before sterilizing of the hydrolysate.

The fermentative products produced in Example 71-73 had a decreased sour taste and a slightly increased bitter taste.

Example 74-76: Preparation of bacteria fermentative products for food containing lactic acid 74-76

The present inventors have produced the bacteria fermentative products with the same method as the above Example 2 except adding 1, 2, 3 weight part of *Atractylodes japonica* root extract to 100 weight part of skim milk-hydrolysate before sterilizing of the hydrolysate.

The fermentative products produced in Example 74-76 had a decreased sour taste and a slightly increased

bitter and astringent taste. The characteristics of the fermentative products produced in Example 71-76 were tested and the results were summarized in Table 16.

<Table 16>

Characteristics of the fermentative products produced in Example 71-76

Index	Amounts of added extracts (weight part) to 100 weight part of hydrolysate					
	Extract of *Ulmus davidiana var. japonica*			Extract of *Atractylodes japonica* root		
	1	2	3	1	2	3
	Example 71	Example 72	Example 73	Example 74	Example 75	Example 76
Concentration of *Lactobacillus* (titer)	8.7×10^{9}	2.2×10^{10}	6.9×10^{9}	4.8×10^{10}	2.0×10^{9}	2.2×10^{9}
Cell morphology	General	General	General	General	General	General
Additional micro flora	No	No	No	No	No	No
Term of preservation (day)	3	3-4	2-3	4	2-3	2-3
Term of validity (day)	17	18	16	20	15	15
Taste, flavor	4.2	4.5	4.2	4.1	4.0	4.0

Example 77-79: Preparation of bacteria fermentative

products for food containing lactic acid 77-79

The present inventors have produced the bacteria fermentative products with the same method as the above Example 3 except adding 1, 2, 3 weight part of *Ulmus davidiana var. japonica* extract to 100 weight part of skim milk-hydrolysate before sterilizing of the hydrolysate.

The fermentative products produced in Example 77-79 had a decreased sour taste and a slightly increased bitter taste.

Example 80-82: Preparation of bacteria fermentative products for food containing lactic acid 80-82

The present inventors have produced the bacteria fermentative products with the same method as the above Example 3 except adding 1, 2, 3 weight part of *Atractylodes japonica* root extract to 100 weight part of skim milk-hydrolysate before sterilizing of the hydrolysate.

The fermentative products produced in Example 80-82 had a decreased sour taste and a slightly increased bitter and astringent taste. The characteristics of the fermentative products produced in Example 77-82 were tested and the results were summarized in Table 17.

<Table 17>

Characteristics of the fermentative products produced in Example 77-82

Index	Amounts of added extracts (weight part) to 100 weight part of hydrolysate					
	Extract of *Ulmus davidiana var. japonica*			Extract of *Atractylodes japonica* root		
	1	2	3	1	2	3
	Example 77	Example 78	Example 79	Example 80	Example 81	Example 82
Concentration of *Lactobacillus* (titer)	3.8×10^{9}	3.4×10^{10}	4.0×10^{9}	5.8×10^{10}	1.1×10^{10}	3.1×10^{9}
Cell morphology	General	General	General	General	General	General
Additional micro flora	No	No	No	No	No	No
Term of preservation (day)	3	3	3	4	3	2-3
Term of validity (day)	15	18	15	20	18	15
Taste, flavor	4.0	4.7	4.0	4.0	4.1	4.1

In the present invention, by adding the extracts of *Ulmus davidiana var. japonica* and *Atractylodes japonica* roots, the strong sour taste of *Lactobacillus* culture becomes mild still with original flavor. When they are added with fewer amounts than 1 weight part to 100 weight part of hydrolysate, they have no effect and

with more than 3 weight part, the taste becomes bitter not to be suitable for food production.

Example 83: Preparation of bacteria fermentative products for food containing lactic acid 83

The present inventors have produced the bacteria fermentative products with the same method as the above Example 1 except using milk instead of skim milk.

Example 84: Preparation of bacteria fermentative products for food containing lactic acid 84

The present inventors have produced the bacteria fermentative products with the same method as the above Example 1 except using soybean milk instead of skim milk.

The fermentative products produced in Example 83 and 84 were brown-colored, sticky solutions having sour taste and oxidative milk-like smell, and contained more than 10^9 *Lactobacillus* per 1 ㎖. After storing for 3 days at 4℃, the original characteristics of the fermentative products were remained. And the sensuous properties of the fermentative products were better than those of fermentative products using skim milk-hydrolysate. The fermentative products produced in Example 83 and 84 were compared with the fermentative products produced in Example 1 and 4, and the results

were summarized in Table 18.

<Table 18>

Characteristics of the fermentative products produced in Example 83, 84, 1 and 4

Index	Example 1	Example 83	Example 84	Example 4
Concentration of *Lactobacillus* (titer)	6.5×10^9	6.6×10^9	5.2×10^9	2.4×10^8
Cell morphology	General	General	General	Degenerated
Additional micro flora	No	No	No	No
Term of preservation (day)	3	3	2-3	1
Term of validity (day)	15-18	15-18	13-16	6-8
Taste, flavor	3.9	4.3	4.3	3.1

Example 85: Preparation of bacteria fermentative products for food containing lactic acid 85

The present inventors have produced the bacteria fermentative products with the same method as the above Example 2 except using milk instead of skim milk.

Example 86: Preparation of bacteria fermentative products for food containing lactic acid 86

The present inventors have produced the bacteria

fermentative products with the same method as the above Example 2 except using soybean milk instead of skim milk.

The fermentative products produced in Example 85 and 86 were brown-colored, sticky solutions having sour taste and oxidative milk-like smell, and contained more than 10^9 *Lactobacillus* per 1 ㎖. After storing for 3 days at 4℃, the original characteristics of the fermentative products were remained. And the sensuous properties of the fermentative products were better than those of fermentative products using skim milk-hydrolysate. The fermentative products produced in Example 85 and 86 were compared with the fermentative products produced in Example 2 and 4, and the results were summarized in Table 19.

<Table 19>

Characteristics of the fermentative products produced in Example 85, 86, 2 and 4

Index	Example 2	Example 85	Example 86	Example 4
Concentration of *Lactobacillus* (titer)	6.7×10^9	6.1×10^9	5.8×10^9	2.4×10^8
Cell morphology	General	General	General	Degenerated
Additional micro flora	No	No	No	No

Term of preservation (day)	3	3	2-3	1
Term of validity (day)	15-18	15-18	13-15	6-8
Taste, flavor	3.9	4.2	4.2	3.1

Example 87: Preparation of bacteria fermentative products for food containing lactic acid 87

The present inventors have produced the bacteria fermentative products with the same method as the above Example 3 except using milk instead of skim milk.

Example 88: Preparation of bacteria fermentative products for food containing lactic acid 88

The present inventors have produced the bacteria fermentative products with the same method as the above Example 3 except using soybean milk instead of skim milk.

The fermentative products produced in Example 87 and 88 were brown-colored, sticky solutions having sour taste and oxidative milk-like smell, and contained more than 10^9 *Lactobacillus* per 1 ㎖. After storing for 3 days at 4℃, the original characteristics of the fermentative products were remained. And the sensuous properties of the fermentative products were better than those of fermentative products using skim milk-

hydrolysate. The fermentative products produced in Example 87 and 88 were compared with the fermentative products produced in Example 3 and 4, and the results were summarized in Table 20.

<Table 20>

Characteristics of the fermentative products produced in Example 87, 88, 3 and 4

Index	Example 3	Example 87	Example 88	Example 4
Concentration of *Lactobacillus* (titer)	6.9×10^9	7.1×10^9	5.2×10^9	2.4×10^8
Cell morphology	General	General	General	Degenerated
Additional micro flora	No	No	No	No
Term of preservation (day)	2-3	3	2-3	1
Term of validity (day)	15-18	15-18	15-16	6-8
Taste, flavor	3.9	4.3	4.2	3.1

As shown above, the sensuous properties such as taste and flavor of the fermentative products of the present invention could be more enhanced by using hydrolysate of milk or soybean milk than using skim milk.

When skim milk-hydrolysate was used to produce

fermentative products, sensuous properties of food were not satisfactory. Thus, it is preferable to produce fermentative products using hydrolysate of milk or soybean milk to upgrade taste and flavor.

From the results of the Examples using hydrolysate of milk or soybean milk, it was also confirmed that the therapeutic characteristics and biological value of the fermentative products produced by using hydrolysate of milk or soybean milk were not different comparing to those produced by using skim milk-hydrolysate and fermentative products excellent in sensuous properties such as taste and flavor could be produced by using hydrolysate of milk or soybean milk.

Fermentative products of the present invention were daily culture of *Bifidobacteria*, *Lactobacteria*, and *thermophile streptococcus* in skim milk-hydrolysate. It is more preferable to culture various bacteria strains together than to culture single bacteria strain only. Therefore, the present inventors analyzed the characteristics and values of fermentative products of the present invention produced by mixing each bacteria strain (*Bifidobacteria*, *Lactobacteria*, and *thermophile streptococcus*) at the required ratio for culture.

Example 89: Preparation of bacteria fermentative

products for food containing lactic acid 89

The present inventors have produced the bacteria fermentative products with the same method as the above Example 1 except using daily cultures of *Bifidobacterium bifidum* (KCTC3202) and *Lactobacillus plantarum* (KCTC1048) together as an inoculum at the ratio of 1:1.

Example 90: Preparation of bacteria fermentative products for food containing lactic acid 90

The present inventors have produced the bacteria fermentative products with the same method as the above Example 1 except using daily cultures of *Bifidobacterium bifidum* (KCTC3202) and *Streptococcus thermophilus* (KCTC3658) together as an inoculum at the ratio of 1:1.

Example 91: Preparation of bacteria fermentative products for food containing lactic acid 91

The present inventors have produced the bacteria fermentative products with the same method as the above Example 1 except using daily cultures of *Lactobacillus plantarum* (KCTC1048) and *Streptococcus thermophilus* (KCTC3658) together as an inoculum at the ratio of 1:1.

Example 92: Preparation of bacteria fermentative

products for food containing lactic acid 92

The present inventors have produced the bacteria fermentative products with the same method as the above Example 1 except using daily cultures of *Bifidobacterium bifidum* (KCTC3202) and *Lactobacillus plantarum* (KCTC1048) together as an inoculum at the ratio of 1:2.

Example 93: Preparation of bacteria fermentative products for food containing lactic acid 93

The present inventors have produced the bacteria fermentative products with the same method as the above Example 1 except using daily cultures of *Bifidobacterium bifidum* (KCTC3202) and *Streptococcus thermophilus* (KCTC3658) together as an inoculum at the ratio of 1:2.

Example 94: Preparation of bacteria fermentative products for food containing lactic acid 94

The present inventors have produced the bacteria fermentative products with the same method as the above Example 1 except using daily cultures of *Bifidobacterium bifidum* (KCTC3202) and *Lactobacillus plantarum* (KCTC1048) together as an inoculum at the ratio of 2:1.

Example 95: Preparation of bacteria fermentative products for food containing lactic acid 95

The present inventors have produced the bacteria fermentative products with the same method as the above Example 1 except using daily cultures of *Lactobacillus plantarum* (KCTC1048) and *Streptococcus thermophilus* (KCTC3658) together as an inoculum at the ratio of 2:1.

Example 96: Preparation of bacteria fermentative products for food containing lactic acid 96

The present inventors have produced the bacteria fermentative products with the same method as the above Example 1 except using daily cultures of *Streptococcus thermophilus* (KCTC3658) and *Lactobacillus plantarum* (KCTC1048) together as an inoculum at the ratio of 2:1.

Example 97: Preparation of bacteria fermentative products for food containing lactic acid 97

The present inventors have produced the bacteria fermentative products with the same method as the above Example 1 except using daily cultures of *Bifidobacterium bifidum* (KCTC3202) and *Streptococcus thermophilus* (KCTC3658) together as an inoculum at the ratio of 2:1.

Example 98: Preparation of bacteria fermentative

products for food containing lactic acid 98

The present inventors have produced the bacteria fermentative products with the same method as the above Example 1 except using daily cultures of *Bifidobacterium bifidum* (KCTC3202), *Lactobacillus plantarum* (KCTC1048) and *Streptococcus thermophilus* (KCTC3658) together as an inoculum at the ratio of 1:1:1.

The below Table 21 is showing the characteristics of the fermentative products produced in Example 89-98, suggesting that the inocula used in this invention has strong resistance against acid and has excellent therapeutic characteristics and biological value. Thus, it is also meaningful to compare and analyze the characteristics of fermentative products according to the used inocula and the mixing ratio thereof.

<Table 21>

Characteristics of the fermentative products produced in Example 89-98

Index	Example 89	Example 90	Example 91	Example 92	Example 92	Example 93	Example 94	Example 95	Example 96	Example 97
	B:L	B:S	L:S	B:L	B:S	B:L	L:S	S:L	B:S	B:L:S
	1:1	1:1	1:1	1:2	1:2	2:1	2:1	2:1	2:1	1:1:1

Concentration of *Lactobacillus* (Titer)	2.4×10^{10}	9.4×10^{9}	6.6×10^{9}	4.8×10^{10}	8.8×10^{9}	1.8×10^{10}	7.5×10^{9}	6.6×10^{9}	1.0×10^{10}	2.2×10^{10}
Cell morphology	General	General	General	General	General	General	General	General	General	General
Additional micro flora	No	No	No	No	No	No	No	No	No	No
Term of preservation (day)	3-4	3	3	4	3	3-4	3	3	3	3
Term of validity (day)	18	15	15	20	15	15-16	15	15	16	18
Taste, flavor	3.9	4.0	4.0	3.8	4.1	3.9	3.9	4.0	4.0	3.8

INDUSTRIAL APPLICABILITY

As shown above, fermentative products of the present invention contain richer nutrition comparing to the conventional fermentative products since the fermentative products of the present invention were fermented by using own media prepared by hydrolysis of dairy resources and adding plenty of nutrients thereto, and the destruction of nutrients was minimized owing to the pasteurization at low temperature. Besides, the fermentative products were confirmed to have high concentration of *Lactobacillus* (titer), be stored for a long time, be used as foods for treatment and

alimentotherapy, and be used as biological food additives. Unlike the conventional oxidized milk products, the fermentative products of the present invention are not sticky which enables for even baby to drink them with a nursing bottle.

Those skilled in the art will appreciate that the concepts and specific embodiments disclosed in the foregoing description may be readily utilized as a basis for modifying or designing other embodiments for carrying out the same purposes of the present invention. Those skilled in the art will also appreciate that such equivalent embodiments do not depart from the spirit and scope of the invention as set forth in the appended claims.

What is claimed is:

1. A method for producing bacteria fermentative media for food containing lactic acid comprising a step of hydrolyzing dairy resources.

2. The method for producing bacteria fermentative media for food containing lactic acid of claim 1, further comprising:
   1) Hydrolyzing dairy resources;
   2) Obtaining filtrate or supernatant by filtration or centrifugation after heating the above hydrolysate;
   3) Adding materials for the growth of bacteria to the above solution; and
   4) Sterilizing the above solution.

3. The method of claim 2, further comprising a step that adding 0.5-5 weight part of starch to 100 weight part of dairy resources before the above step 1 of claim 2.

4. The method of claim 2, wherein one or more dairy resources selected from a group consisting of skim milk, milk, and soybean milk are used.

5. The method of claim 2, wherein the hydrolysis is performed by adding one or more enzymes selected from a group consisting of glycolyase, protease, and pancreatin.

6. The method of claim 2, wherein one or more materials for the growth of bacteria selected from a group consisting of agar, NaCl, yeast extract, peptone, lactose, and dextrose are used.

7. The method of claim 2, wherein further adding 0.1-10 weight part of hydrolyzed/concentrated amino acid mixture to 100 weight part of the hydrolysate of the above step 1.

8. The method of claim 2, wherein further adding 0.1-10 weight part of one or more components selected from a group consisting of pine needles extract, honey, and mixture of honey and pine needles extract (honey:pine needles extract = 0.5:2.0-1.0:2.0) to the hydrolysate of the above step 1.

9. The method of claim 2, wherein further adding 0.1-10 weight part of extracts of *Ulmus davidiana var. japonica* or *Atractylodes japonica* roots to the 100 weight part of the hydrolysate of the step 1.

10. The method of claim 2, wherein the sterilizing of step 4 is performed at 110-118℃.

11. The method for producing bacteria fermentative media for food containing lactic acid of claim 2, comprising the following steps:

1) Add 0.5-5 weight part of starch to 100 weight part of dairy resources followed by stirring, after which heat thereof at 95-105℃ for 5-60 minutes;

2) Control the pH of the above solution to 6.0-8.0 and cool thereof at 75-85℃, after which add 0.001-0.003 weight part of amylase thereto;

3) Cool the above solution to 45-50℃, after which add 0.05-0.2 weight part of protease thereto followed by hydrolyzing;

4) Control the pH of the above solution to 4.4-4.6 and then heat thereof at 95-105℃ for 10-20 minutes, after which remove precipitates by filtration or centrifugation to obtain supernatants;

5) Add 0.5-1.5 volumes of distilled water to the above supernatants for dilution, and then add materials necessary for the growth of bacteria thereto; and

6) Sterilize the above media.

12. The method for producing bacteria fermentative media for food containing lactic acid of claim 2, comprising the following steps:

1) Stir the dairy resources, after which heat thereof at 95-105℃ for 5-60 minutes;

2) Cool the above solution to 40-50℃, after which control the pH to the level of 7.5-8.5;

3) Add 0.05-0.2 weight part of pancreatin to the above solution followed by hydrolyzing;

4) Control the pH of the above hydrolysate to the level of 4.4-4.6, and then heat thereof at 95-105℃ for 10-20 minutes, after which remove precipitates by filtration or centrifugation to obtain supernatants;

5) Add 0.5-1.5 volumes of distilled water to the above supernatants for dilution, and then add materials necessary for the growth of bacteria thereto; and

6) Sterilize the above media.

13. A bacteria fermentative media for food containing lactic acid produced by the method of any one of claim 1-12.

14. A method for producing bacteria fermentative products for food containing lactic acid comprising a step of inoculating bacteria fermentative media for food containing lactic acid of claim 13 with bacteria and culturing thereof.

15. The method for producing bacteria fermentative products for food containing lactic acid of claim 14, wherein the bacteria is selected from a group consisting of *Lactobacterium plantarum* 8 PA3, *Lactobacter plantarum* 296, *Lactobacillus acidophilus*, *Bifidum longum* 379 M, *Bifidum* 791, *Bifidum* 1, *Streptococcus thermophills*, *Lactobacillus plantarum* (KCTC1048), *L. acidophilus* (KCTC 3111), *Bifidobacterium bifidum* (KCTC 3202, 3357), *B. longum* (KCTC 3421, 3128), *Streptococcus thermophilus* (KCTC 3658), *Bifidobacterium longum* 379M ПартИЯ1 ОТ 11.00.01г., *Lactobacillus acidophilus* ПартИЯ2 ОТ 10.02.01г.

16. The method for producing bacteria fermentative products for food containing lactic acid of claim 14, wherein inoculating 100 weight part of fermentative media with 3-10 weight part of bacteria.

17. The method for producing bacteria fermentative products for food containing lactic acid of claim 14, wherein the culture is performed for 10-25 hours at 36-38℃ in a fermentor after inoculation with bacteria.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR02/01114

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 C12N 1/20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7 C12N 1/20

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the internationl search (name of data base and, where practicable, search terms used)

CA, Delphiom, "milk hydrolyzate", "medium", "lactic acid bacteria"

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Karlikanova, S.N. et al., "Endo, a dehydrated nutrient medium from dairy by-products", Molochn. Prom-st., 12, 23-5, 1983. See abstract.	1-7, 10-17
X; A	Ervol'der, T.M. et al., "Action of enzymic hydrolyzates on the accumulation of the biomass of bipidobacteria", Molochn. Prom-st., 12, 15-17, 1980. See abstract.	1-7, 10-17; 8, 9
X	Gomes, Ana M.P., et al., "Growth enhancement of Bipidobacterium lactis Bo and Lactobacillus acidophilus Ki by milk hydrolyzates", :J. Dairy Sci., 81(11), 2817-25, 1998. See abstract.	1-7, 10-17
X	Said, M.R., et al., "Acceleration of white soft cheese ripening by using some bacterial growth factors", Egypt J. Dairy Sci., 12(2), 219-29, 2984. See abstract.	1-7, 10-17
X; A	JP 03240436 A2 (Snow Brand Milk Products Co., Ltd.), 25 Oct. 1991.	1-7, 10-17; 8, 9
X; A	JP 08238066 A2 (Fuji Oil Co., Ltd.), 17 Sep. 1998.	1-7, 10-17; 8, 9
Y	DE 3523148 A1 (Tihole, F.), 08 Jan. 1987.	1-7, 10-17

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevence

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevence; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevence; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
09 OCTOBER 2002 (09.10.2002)	09 OCTOBER 2002 (09.10.2002)

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR02/01114

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 03240436 A2	25 Oct. 1991	JP 02849943 B2	27 Jan. 1999
JP 08238066 A2	17 Sep. 1998	none	
DE 3523148 A1	08 Jan. 1987	none	